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Recombinant Elastin-Like Polymers: from the design towards application

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Abstract

With the development of protein engineering and nano(bio)technologies it is now possible to use amino acids to design and produce genetically engineered Protein-Based Polymers (PBPs).
These polymers occur in a wide range of biological systems, fulfilling precise functional roles. Its properties are due to the presence of short repeating sequences contained in the fibrous proteins, such as mammalian elastin. Elastin-Like Polymers (ELPs) are biopolymers based on the aminoacid sequence VPGXG (where X is any naturally occurring aminoacid except proline) that reversibly coacervate above a critical temperature (T_t), showing a visible transition phase that can be explored as a purification method. Additionally, the ability of ELPs to self-assemble into nanostructures in response to environmental signals allows them to be explored for controlled drug delivery devices or nanosensors. The polymer poly(VPAVG), a ELP where the central glycine (G) is substituted by a L-alanine (A), was chemically synthesized by Rodríguez-Cabello and co-workers and described by Urry as having thermoplastic properties. These groups reported its characterization, demonstrating its extreme biocompatibility both *in vitro* and *in vivo,* as well as the ability to self-assemble, forming microparticles that can entrap active substances during the self-assembling process.
In the present work a new thermally responsive, biologically synthesized ELP based on the $(VPAVG)_{220}$ sequence was produced with standard molecular genetic tools and, as expected, the polymer displayed an inverse temperature transition (T_t) which could be explored as a purification approach (1). Sequence and purity was confirmed by MALDI TOF and SDS-PAGE analysis and purified polymer was thermally and physically characterized. Due to its self-assembling behaviour near 34 ºC stable spherical microparticles of a ~1 µm diameter were obtained, ready solubilized when a strong undercooling was achieved. By fusing the ELP with Subtilisin E DNA sequence we were able to produce a soluble chimeric protein with improved properties in wool yarn treatment, when compared with the commercial Subtilisin (2). The ELP was also exploited as a drug delivery system for the controlled release of BMP-2 and BMP-14 (3). The ELP system showed a high efficiency of encapsulation with a sustained release for 14 days. The activity of the growth factors was maintained and an increased bioactivity was observed when combining the release of BMP-2 and BMP-14.

1 – Machado, R., Ribeiro, A.J., Padrão, J., Silva, D., Nobre, A., Teixeira, J.A., Arias, F.J., Cunha, A.M., Rodríguez-Cabello, J.C., Casal, M., Journal of NanoResearch, 2009, 6, 133-145.
2 – Araújo, R., Silva, C., Machado, R., Casal, M., Cunha, A.M., Rodríguez-Cabello, J.C., Cavaco-Paulo, A., Biomacromolecules, 2009, 10, 1655-1661.
3 – Bessa, P.C., Machado, R., Nürnberger, S., Dopler, D., Banerjee, A., Cunha, A.M., Rodríguez-Cabello, J.C., Redl, H., van Griensven, M., Reis, R.L., Casal, M., submitted.